Determination of (+)-Catechin and Antioxidant Activity in Faloak (Sterculia quadrifida R.Br) Stem Bark Infusion

by Audrey Gracelia Riwu

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Research Paper

Determination of (+)-Catechin and Antioxidant Activity in Faloak (*Sterculia quadrifida* R. Br) Stem Bark Infusion

Audrey Gracelia Riwu[1], Jusak Nugraha[2], Djoko Agus Purwanto[3*], Erwin Astha Triyono[4]

[1]*Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, 60132 Indonesia*
[2]*Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, 60132, Indonesia*
[3]*Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, 60115, Indonesia*
[4]*Department of Internal Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, 60132, Indonesia*
***Corresponding author**: djokoagus@ff.unair.ac.id

Abstract

Oxidative stress is a condition that can damage human cells and tissues and has been linked to a number of illnesses, including cancer, cardiovascular disease, autoimmune disorders, and neurological diseases. Oxidative stress conditions can be brought on by pollution, radiation exposure, and an unhealthy lifestyle. Antioxidants are substances that can be used to both prevent and treat oxidative stress. This study aimed to identify and quantify (+)-catechin levels and antioxidant activity of the stem bark of *Sterculia quadrifida* R. Br extracted by the infusion method, a method similar to traditional medicine processing generally in the community. Determination of (+)-catechin and antioxidant activity of *S. quadrifida* were evaluated by HPLC and DPPH assay, respectively. Quantification of (+) catechin content by HPLC system with wavelength 280 nm and antioxidant activity by spectrophotometry method with wavelength 517 nm. The results show that the mean value of (+)-catechin level was 7.786% and the IC50 value of the antioxidant activity was 51.5 μg/mL having a moderate antioxidant activity category. *S. quadrifida* stem bark infusion can be utilized as a medication candidate for the prevention or treatment of a variety of disorders caused by oxidative stress.

Keywords

Sterculia quadrifida R. Br, Faloak, (+)-Catechin, Antioxidant Activity, HPLC, DPPH

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1. INTRODUCTION

Cancer, cardiovascular disease, autoimmune disorder, and cardiovascular and neurodegenerative disease (i.e Alzheimer's disease and Parkinson's disease) are widely known to have a correlation with oxidative stress. Oxidative stress is a condition due to an imbalance between the increase of ROS and RNS secretion that leads to cell and tissue damage. There are many factors that contribute to this condition like exposure to air pollution, tobacco smoke, ozone, alcohol, cooking (used oil, fat, and smoked meat), and another unhealthy lifestyle. These exogenous substances are metabolized or broken down into free radicals after entering the body through various mechanisms (Pizzino et al., 2017). Free radicals are substances (atoms, ions, or molecules) that contain one or more unpaired electrons that interfere with or attack nearby electrons in order to form bonds with them, which causes oxidative stress (Di Meo and Venditti, 2020).

Antioxidants are one strategy for reducing oxidative stress. By giving electrons to free radicals, antioxidants are able to neutralize them and inhibit cellular damage potential due to oxidative stress. Antioxidants can be found in both manufactured and natural goods, although generally, natural antioxidants are preferred because they have less of an impact on the body. Some sources of natural antioxidants are usually derived from plant materials, such as vegetables, fruits and herbs (Nimse and Pal, 2015; Lourenço et al., 2019). (+)-Catechin is one of the naturally occurring antioxidants, a derivative form of catechin compound, which is mostly found in woody plants (Yamaji and Ichihara, 2012). (+)-Catechin is a flavonoid-derived flavan-3-ols compound that is known to have anticarcinogenic, anti-tumor, antibiotic, and antioxidant activity which makes it essential in treating various diseases (Lee et al., 2020; Musial et al., 2020).

Sterculia quadrifida R. Br (world's known name: peanut tree/kurrajong; local name in Indonesia: Faloak) is one of the woody plants that belong to the *Malvaceae* family that can be used as a source of natural antioxidants because it is found to have a variety of secondary metabolites, including flavonoids, alkaloids, terpenoids, saponin and phenolic (Tenda et al., 2021;

Siswadi et al., 2021). This plant can be found in plentiful supply from Queensland (Western Australia), Papua New Guinea, Timor Leste, and Indonesia (Govaerts et al., 2021; Siswadi et al., 2021). The stem bark of trees, which is processed through boiling or decoction, is the most widely utilized plant part in Indonesia (Tenda et al., 2021).

Traditional medicine can be utilized in a variety of ways and with different types of solvents, but in the community, boiling with water is the approach that is most commonly applied. The process of boiling water is thought to be the simplest and safest one that can be used in regular community life (Keskin, 2018; Wachtel Galor and Benzie, 2011).

Based on the above background, the aim of this study is to determine and quantify (+)-catechin using HPLC, followed by antioxidant activity using DPPH methods in the infusion of *S. quadrifida* stem bark. For additional. in this study, we also identify the functional group of compounds with FTIR spectrophotometry methods. The infusion method is an extraction technique that is similar to the community's processing technique, which involves heating water. Therefore, this study is expected to provide an overview of the (+)-catechin levels and antioxidant activity found in traditional medicines that are processed in a common way in the community.

2. EXPERIMENTAL SECTION

2.1 Materials

S. quadrifida stem bark was taken from the tree which grows in the city of Kupang, East Nusa Tenggara, Indonesia. Determination of these plants was carried out at the Indonesian Institute of Sciences, Biological Research Centre, Cibinong, Bogor. The determination process was conducted on these plant parts such as seeds, flowers, leaves, fruit, and stem bark. The (+)-catechin standard was obtained through Sigma Aldrich, which was interpreted by the Testing Services Unit of the Faculty of Pharmacy, Airlangga University, Indonesia.

2.2 Extraction

S. quadrifida stem bark was extracted using the infusion method in order to represent the traditional method of processing by the community. The stem bark of this plant was gathered, sorted, crushed, and mashed using a blender in quantities of up to 500 grams. Aquades solvent was used to dissolve the mashed simplisia, which was then heated at 90°C for 15 minutes. After 15 minutes, the filtrate is retrieved, gathered, and dried using the freeze-drying method to produce up to 16 grams of dry powdered stem bark (Azwanida, 2015).

2.3 Preparation of Samples and Standard Solutions

The powdered *S. quadrifida* stem bark was taken as much as 500 mg and put into a flask with a volume of 50 ml. Then added ethanol and water with a ratio of 1: 1 until exactly according to the mark on the flask. The sample then underwent sonication for 15 minutes and was filtered with a nylon membrane of 0.45 microns into the HPLC vial and analyzed. A raw stock solution of catechins of 10.4 mg was dissolved in 10 ml of methanol which is then filtered using 0.2 microns of nylon (Gottumukkala et al., 2014).

2.4 Calibration

The standard calibration process was carried out by diluting the catechin stock solution with methanol with a concentration range of 200-1000μL. The standard curve was obtained using the peak area of five concentrations in five repeat tests and expressed by a linear-quadratic regression equation Gottumukkala et al. (2014).

2.5 FTIR Analysis

Fourier-transform infrared spectroscopy (FTIR) is a technique used to obtain the infrared spectrum of the absorption or emission of a solid, liquid, or gas. The FTIR spectrometer simultaneously collects high-resolution spectra data over a wide spectrum range. FTIR method is commonly used to identify the groups present in a compound or group of compounds that describe the identity of the natural product extract under study. Therefore, FTIR is important to provide an overview of the characteristics of a particular plant extract (Pharmawati and Wrasiati, 2020). Approximately 10 mg of water extract of *S. quadrifida* stem bark was mixed with 100 mg of KBr and packaged into sample pellets and then read by the FTIR spectrophotometer (Perkin Elmer, 1302F6802). The results obtained were analyzed by their functional groups starting from wave numbers 450 cm^{-1} to 4000 cm^{-1} (Pakkirisamy et al., 2017).

2.6 Determination of (+)-catechin

The (+)-catechin analysis process is carried out using the HPLC Agilent 1100 system consisting of a quaternary pump, autosampler DAD detector, column compartment, and degasser with Merck Lichrosper 100 RP-18, 250 x 4 mm, 0.5 μ column. The entire analysis process is carried out at a temperature of 25°C with a mobile phase using a solution of acetonitrile (A) and methanol (B) with a gradient of 1.2 mL/minute. The mobile phase is filtered using nylon 0.2 microns where before using the gas was removed first. The sample injected into HPLC was 20 μL with a wavelength (λ) of 280 nm. Chromatography peaks are identified by comparing retention times between analytes with standards. Quantification was determined by peak integration using external standard methods (Roman and Solomiia, 2019).

2.7 Antioxidant Activity

The antioxidant activity of the sample was tested using the DPPH method with ascorbic acid as a control. *S. quadrifida* stem bark infusion samples and ascorbic acid as standard were dissolved with ethanol solvents and made in several concentrations. Each solution is picked as much as 1 mL to be added to 3 mL of DPPH solution 40 ppm in ethanol. Then the solution is shaken firmly and let stand at room temperature for 30 minutes. Then the absorbance was measured using a spectrophotometer at a maximum wavelength (λ) of 517 nm. These tests were

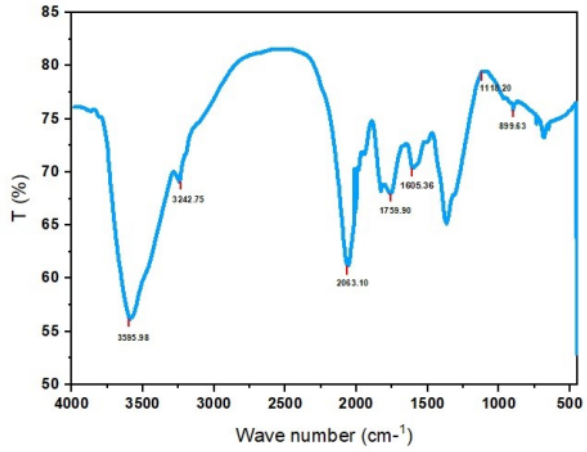

Figure 1. FTIR Spectrum of *S. quadrifida* Stem Bark

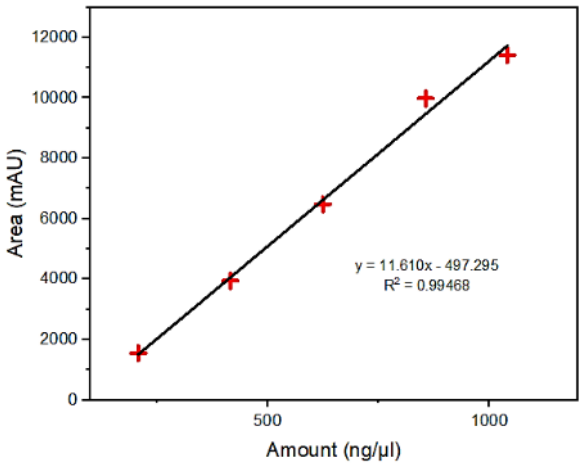

Figure 2. (+)-Catechin Standard Calibration Curve

repeated twice. Radical scavenging activity is indicated as the presentation of free radical inhibitors by a sample calculated with the formula:

$$\text{DPPH}_{scavenging\,activity}(\%) = \frac{\text{ABS}_{control}\text{-ABS}_{sample}}{\text{ABS}_{control}} \times 100\% \quad (1)$$

Where Abscontrol is the absorbance of the DPPH and Abssample is the absorbance of the sample. The concentration of the sample and the percent inhibition obtained were plotted on the x and y axis respectively in the linear regression equation y=a+bx. This equation is used to determine the IC50 value of the sample. The IC50 value is the sample concentration that can reduce DPPH radicals by 50% of the initial concentration. The IC50 value is obtained from the x value (concentration) after replacing the y value with 50 (50% inhibition). The interpretation of IC50 results is that the lower the absorbance of mixed reactions indicates that the higher the activity of free radicals (Allam et al., 2020; Sinala et al., 2020).

3. RESULT AND DISCUSSION

3.1 FTIR Analysis

The functional groups in *S. quadrifida* stem bark extract was predicted by interpreting the infrared absorption spectra. The FTIR spectrum result can be seen in Figure 1, and the interpretation of the functional group can be seen in Table 1.

Based on the results of FTIR analysis, seven compounds were found in the stem bark infusion of *S. quadirifda*, namely alcohol, poly hydroxyl compound, isothiocyanate, aromatic compound, ketone compound, secondary alcohol, and aromatic phosphates.

3.2 Determination of (+)-catechin

Calibration of the (+)-catechin standard used at concentrations of 200 μL – 1000 μL (208, 416, 624, 832, 1040 ppm) indicates good linearity which means the correlation coefficient of the equation was close to 1 and the calibrations curves lead straight lines with a wide range (Gottumukkala et al., 2014; Pinto et al., 2017). The (+)-catechin content in the *S. quadrifida* stem bark infusion was calculated using the linear regression equation obtained by the curve that can be seen in Figure 2. Chromatograms of the identification of the standard solutions and infusions can be seen in Figures 3 and 4. The result of the determination of (+)-catechins levels can be seen in Table 2.

Based on the results of the HPLC test, it is found that the average result of (+)-catechins found in the infusion of the *S. quadrifida* stem bark is 7.786 % w/w with an RPD of 0.889% (Table 2). Studies on the quantification of (+)-catechin in the stem bark of *S. quadrifida* have never been done before in previous studies. Therefore, the determination of (+)-catechin levels in this study is also a novelty in the exploration of specific compounds in the stem bark of *S. quadrfida*.

(+)-Catechin is a derivative form of catechin, polyphenols *flavan-3-ols* that belong to the flavonoid family. This compound is composed of two aromatic rings and several hydroxyl groups, which are classified into two groups: free catechin and esterified catechins. This compound has a molecular weight of 290.27 g/mol with the molecular formula $C_{15}H_{14}O_6$ (Figure 5) (Diniyah et al., 2022; Shukla et al., 2018). (+)-Catechin and other catechin derivatives have been shown to have neuroprotective effects as well as antioxidant, anti-cancer, and antimicrobial (Chang and Wu, 2011).

Quantification of (+)-catechin in *S. quadrifida* stem bark has never been done in previous studies. However, several studies regarding the quantification of (+)-catechin in other plants have been carried out. A study conducted by Silva et al. (2017) stated that the catechin levels found in the spray-dried extract (ethanol 95%) of *Pimenta pseudocaryophyllus* was 5.44%. A study conducted by Nurliayana et al. (2016) on the methanol, dichloromethanen, and hexane extracts of *Uncaria*

Table 1. TIR Spectrum Values and Functional Groups of *S. quadrifida* Stem Bark

No	Wave Number (cm^{-1})	Reference Wave Number (cm^{-1})	Functional Group Assignment	Phyto-compounds Identified
1	3595	3700-3584	OH stretch	Alcohol
2	3242	3570-3200	O-H stretch, Hydroxy group, H-bonded	Poly Hydroxy compound
3	2063	2140-1990	N=C=S stretch	Isothiocyanate
4	1759	2000-1650	C-H bend	Aromatic compound
5	1605	1650-1600	C=O stretching vibration, ketone group	Ketone compound
6	1118	1150-1085	C-O stretch	Secondary alcohol
7	899	995-85	P-O-C stretch	Aromatic phosphates

Table 2. Determination Results of (+)-Catechin Levels and Antioxidant Activity in *S. quadrifida* Stem Bark Infusion

Catechin Levels			Antioxidant Activity (IC50)		
Mean (% w/w)	SD (%)	RPD (%)	Mean (μg/mL)	SD (%)	RPD (%)
7.786	0.049	0.889	51.5	0.29	0.8

Note: SD: Standard Deviation; RPD: relative percentage differences

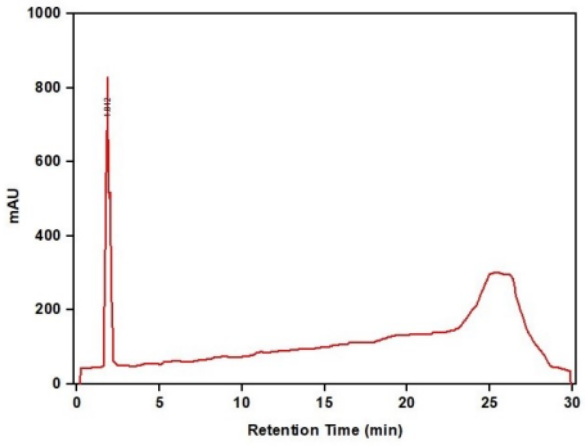

Figure 3. HPLC Chromatogram of (+)-Catechin Standard Solution. Retention Time at 1.812 Min.

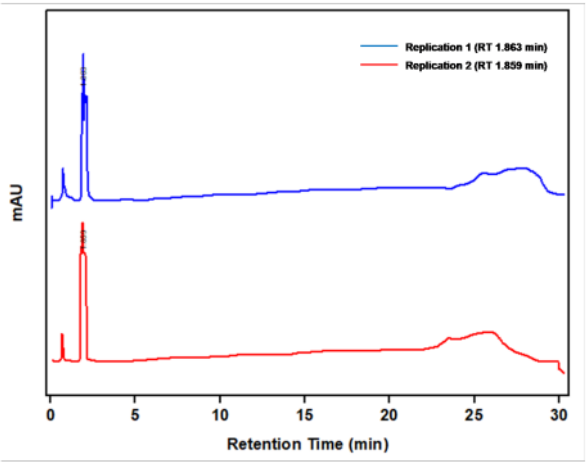

Figure 4. Chromatogram of (+)-Catechin in *S. quadrifida* Stem Bark Infusion

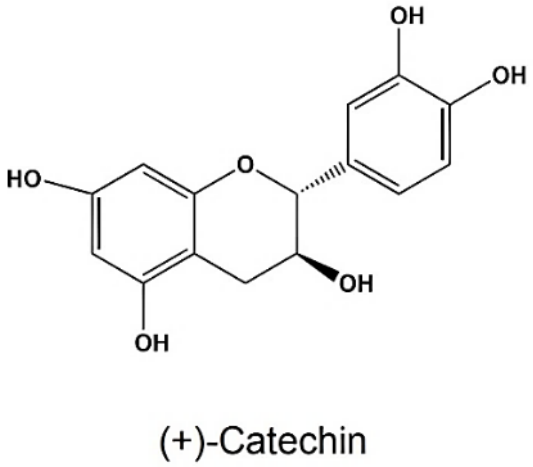

Figure 5. Molecular Structure of (+)-Catechin (Isemura, 2019)

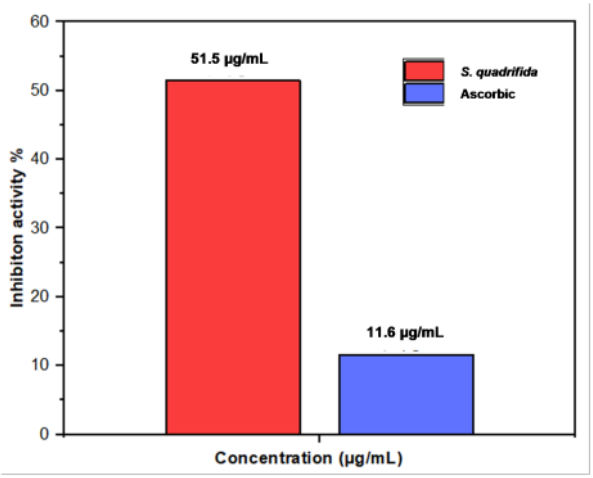

Figure 6. Differences of Antioxidant Activity Between *S. quadrifida* Stem Bark Infusion and Ascorbic Acid as a Control

gambir (Hunter) Roxb stem bark was 5.12%, 0.92%, respectively, and no catechins were found in the hexane extract. Based on the two studies above, it can be said that the differences in catechin levels found were caused by differences in plant species and solvents used in each study.

A study conducted by Koch et al. (2018) showed that the levels of (+)-catechin in black tea, green tea, and white tea products from Kenyan germplasm were 0.30%, 0.39%, and 0.57%, respectively. Meanwhile, in green tea products from China and Japan germplasms were 0.13% and 0.12%. Based on these results, it was found that (+)-catechin levels in tea products were found to be lower when compared to (+)-catechin levels in *S. quadrifida* stem bark infusion. This is a reasonable, because, in tea, the level of (+)-catechin has the lowest level when compared to other catechins (Koch et al., 2018; Singh et al., 2011). Catechins are the most abundant polyphenols in tea which account for 70-80% of the total polyphenols. There are four types of catechins that are most dominant in tea, namely (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-O-gallate (ECG), and (-)-epigallocatechin -3-O-gallate (EGCG), where of the four types of catechins, the concentration of EGCG dominates as much as 50-80% of the total catechins (Koch et al., 2018; Sivanesan et al., 2022). Therefore, further studies are needed to identify the total catechins in the stem bark of *S. quadrifida* so that further comparisons can be made.

3.3 Antioxidant Activity

Results from the DPPH assay using the spectrophotometry method of *S. quadrifida* stem bark infusion can be seen in Table 1. The test also was done by replicating twice because both of the results were very close to each other. The differences in antioxidant activity between *S. quadrifida* stem bark and ascorbic acid can be seen in Figure 6.

Based on the IC50 value, antioxidant activity is divided into five categories, namely very strong (<10 µg/mL), strong (10-50 µg/mL), moderately (50-100 µg/mL), and weak (100-250 µg/mL), and inactive (> 250 µg/mL) (Roy and Dutta, 2021). This shows that the lower the IC50 value, the higher the antioxidant activity. According to the classification, *S. quadrifida* stem bark infusion is classified in the moderate category because the IC50 value is 51.5 µg/mL, while ascorbic acid, which has an IC50 value of 11.6 µg/mL is classified in the strong category (Figure 6). This is presumably because the stem bark of *S. quadrifida* contains a variety of compounds compared to ascorbic acid which is a pure compound.

The antioxidant activity of *S. quadrifida* stem bark has been reported in several previous studies. Research conducted by (Saragih and Siswadi, 2019) on ethanol extract found IC50 values from various parts of *S. quadrifida* bark (branch bark, root bark, old regrown bark, new regrown bark, non-striped stem bark) are all below 50 µg/mL, and studies conducted by Dillak et al. (2019) on ethanol extract also obtained IC50 values of 14.17 µg/mL. This is thought to be due to different types of solvents, where the extraction process using ethanol solvents is the best method of knowing antioxidant activity when compared to water solvents (Hidalgo and Almajano, 2017).

The extraction method also has an impact on antioxidant activity. High-temperature extraction methods have the potential to degrade some of the most controllable molecules that affect antioxidant activity. Infusion methods with high temperatures reaching 90°C can have an impact on the outcome since, in general, heating induces a quick initiation reaction and reduces antioxidant activity (Xu et al., 2017; Réblová, 2012). According to a study done in Réblová (2012), there is a linkage between rising temperatures and decreased antioxidant activ-

ity. Antioxidant activity of the easily oxidized antioxidants was decreased at higher temperatures compared to lower temperatures caused by a reduction in the ability of antioxidants to interact with free radicals.

Catechin levels and antioxidant activity are basically related. Catechins are able to counteract free radicals by giving an electron from the phenolic OH group which can reduce free radicals (Bernatoniene and Kopustinskiene, 2018). Catechins can also reduce oxidation by playing a role in chelating and binding metal ions which can start the oxidation process (Nain et al., 2022). Catechins and antioxidants have a relationship with each other, where the higher the concentration of catechins, the higher the level of antioxidants (Lee et al., 2014; Musial et al., 2020; Bartoszek et al., 2018). However, both of them are also influenced by various factors, such as the extraction process, the type of solution, temperature, and the concentration of other compounds in it (Saklar et al., 2015; Hidalgo and Almajano, 2017).

Due to the high-temperature extraction method, and the type of solution used in this investigation, it was discovered that the catechin levels and antioxidant activity were different (depending on the type of plant and solvent) than in the prior study. Nevertheless, extraction with infusion method is seen as being safer, more practical, and more accessible to the community.

4. CONCLUSION

(+)-Catechin has been detected and quantified in *S. quadrifida* stem bark infusion with a level of 7.786%. A moderate level of antioxidant activity in this plant's stem bark was also discovered in this study, with an IC50 value of 51.5 μg/mL. These findings provide a brief overview of the (+)-catechin content and antioxidant activity of *S. quadrifida* stem bark which is processed by the infusion method which is common in the community. Therefore, *S. quadrifida* can be utilized as a medication candidate for the prevention or treatment of a variety of disorders caused by oxidative stress.

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